

Excretion of Phosphoric Acid by Red Imported Fire Ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae)

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ABSTRACT Food sources, mineral redistribution, and excreta are some of the ways ants enrich their mound environment. Phosphorus excretion has not been reported in red imported fire ants, *Solenopsis invicta* Buren. Quantification of phosphoric acid in worker fecal droplets and larval anal liquid of *S. invicta* was performed. Phosphoric acid was trimethylsilylated using *N,O*-bis(trimethylsilyl)trifluoroacetamide, and the tris(trimethylsilyl) phosphate was analyzed using gas chromatography-mass spectrometry. Phosphoric acid made up $2.13 \pm 1.08\%$ (SD) of the dry weight of worker fecal droplets and $2.09 \pm 0.72\%$ of the larval anal liquid. The rate of phosphoric acid production in workers was $0.08 \pm 0.024 \mu\text{g}/\text{ant}/\text{d}$. Because the hindgut of red imported fire ant larva is separated from the midgut, the excretory products in larval anal liquid are primarily of metabolic origin. The presence of phosphoric acid in larval anal liquid shows that red imported fire ants excrete phosphoric acid.

KEY WORDS excretion, worker fecal droplet, larval anal liquid, phosphoric acid

ENRICHMENT OF PHOSPHORUS AND other elements in mound material of the red imported fire ant, *Solenopsis invicta* Buren, has been reported (Herzog et al. 1976, Lockaby and Adams 1985, Green et al. 1998, 1999). Plant and food remains, redistribution of minerals, and ant excreta are all possible sources of increased phosphorus and other elements in nest soil (Herzog et al. 1976, Green et al. 1998, 1999). Such element enrichment also has been found in other ant species, such as the western harvest ant, *Pogonomyrmex occidentalis* Cresson (Carlson and Whitford 1991) and *Lasius niger* Foerster (Frouz et al. 2003). There is no information available on forms and quantity of phosphorus excretion in red imported fire ants. This is surprising in view of its agricultural and medical importance. In an ant colony, excretory products are not just metabolic waste; they often affect colony functions (Hölldobler and Wilson 1990). Excretory phosphorus may have profound effects on biota in ant nests by changing nutrient constituents and pH value. Such chemical modification may be in fact beneficial to the survival and growth of the colony itself. Knowledge of excretory chemistry is critical to understanding the mechanisms by which red imported fire ants modify their environment.

My preliminary chemical analysis of imported fire ant worker fecal droplets indicated a significant amount of phosphoric acid, which is thus a form of

phosphorus excretion for *S. invicta*. The objective of this study was to further evaluate phosphoric acid excretion in *S. invicta*. In addition to worker fecal droplets, larval anal liquid also was analyzed for the presence of phosphoric acid. In red imported fire ant larvae, the hindgut is separated from the midgut (Petrallia et al., 1982, Vinson 1997). Because the alimentary canal is discontinuous, the excretory products in larval anal liquids are primarily of metabolic origin. This presents us with an opportunity to study phosphoric acid excretion of red imported fire ant larvae without having to deal with possible contamination from their food. The results of this study provide us with baseline information on fire ant phosphorus excretion.

Materials and Methods

Insects. Eleven red imported fire ant colonies were used in this experiment. Six colonies were collected from Pearl River County, MS, on 25 March 2005 and the other five were collected from Washington County, MS, on 29 March 2005. Collected colonies were separated by at least 30.5 m. Colonies with mound material were placed in 19-liter plastic buckets coated with Equate mild baby powder (Cumberland Swan Holdings, Smyrna, TN). Ants were separated from soil using the water-drip method (Banks et al. 1981). Ants were maintained in 44.5 by 60.0 by 13.0-cm plastic trays with the inside wall coated with Fluon (Ag Fluoropolymers, Chadds Ford, PA) to prevent escape. Distilled water and *Heliothis zea* pupae were provided to colonies ad libitum. Distilled water was

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provided in 12-cm test tubes with openings plugged with cotton wicks. A 14 by 2.0-cm petri dish, with 1.0 cm of hardened dental plaster (Castone; Dentsply International, York, PA) on the bottom, was placed in the center of each tray. A 5.0-cm-diameter brood chamber was made in the center of the petri dish. Two 8-mm access holes were drilled in the sides of the petri dish. The petri dish lid was painted black (1302 Gloss Black Spray Enamel; Progress Paint Mfg. Co., Inc., Louisville, KY) to block the light. All colonies were maintained at 21–27°C and relative humidity between 50 and 80% with a 12:12 (L:D) photoperiod. All colonies were maintained in the laboratory <1 mo before they were used in any experiments.

Collection of Worker Fecal Droplets. A 7-cm filter paper disc (Whatman, Qualitative 1) in an 8.5 by 2.0-cm petri dish was used to collect worker fecal droplets from each of 11 colonies. The filter paper disc was dried in a dessicator for 24 h and weighed. Workers (0.5 g) were counted and placed on the filter paper disc, which was covered by a 5.5-cm glass petri dish lid. Fire ant workers inside the petri dish lid preferentially deposited fecal droplets on the filter paper along the edge of the lid. The filter paper was collected after 24 h, dried in a dessicator for 24 h, and weighed. Each fecal droplet spot on the filter paper disc was collected by cutting the spot from the filter paper using scissors, and any masticated filter paper particles were also collected. Any fecal droplets on the petri dish lid were collected by scratching them off with a fine knife. Collected material was placed in a 2-ml vial that was capped. Each sample averaged 1.68 ± 0.78 (SD) mg (dry weight). Only one sample was collected from each colony, so there was a total of 11 samples.

Collection of Larval Anal Liquid. Petralia et al. (1982) found two types of anal liquids: a white droplet that contained uric acid and a clear droplet that consisted of water and salt. The presence of uric acid in the white droplet indicates that it is the true excretory product. Therefore, only white anal liquid was collected in this experiment. Reproductive larvae, which develop into winged male and female alates, were used, because they are relatively large and thus easier to handle. Collection of anal liquid followed the method developed by Petralia et al. (1982). Touching the ventral region of a larva with a forceps stimulated it to release a drop of white anal liquid, which was collected with a 5- μ l disposable capillary micropipette (Drummond Scientific Company, Broomall, PA). Each sample, collected from 10–20 larvae, was transferred into a 200- μ l insert in a 2-ml vial, dried in a dessicator for 24 h, and weighed. Each sample averaged 1.96 ± 0.48 mg (dry weight). One sample was collected from each colony. A total of five colonies were used for this experiment, so there was a total of five samples.

Sample Preparation for Gas Chromatography-Mass Spectrometry Analysis. For worker fecal droplets, 50 μ l BSTFA (*N,O*-Bis(trimethylsilyl)trifluoroacetamide) (Sigma-Aldrich, St. Louis, MO) and 200 μ l

hexane were added to the sample. For larval anal fecal liquid, 10 μ l BSTFA and 10 μ l hexane were added. The sample was heated to 60°C for 1 h to facilitate derivatization.

Gas Chromatography-Mass Spectrometry. A Varian gas chromatography-mass spectrometry (GC-MS) system was used for this study. It consisted of a CP-3800 gas chromatograph and a Saturn 2000 mass selective detector, controlled by a Mass Spectrometry WorkStation Version 6.41 (Varian, Walnut Creek, CA). A 30-m-long by 0.25-mm i.d. FactorFour VF-5 MS capillary column with 0.25- μ m film thickness was used (Varian). The GC temperature program was as follows: initial temperature was held at 50°C for 1 min, increased to 250°C at a rate of 20°C/min, and held for 40 min. The split ratio was 1:10, injection temperature was 250°C, and transfer line temperature was 200°C. Helium was used as the carrier gas, and the flow rate was 1.0 ml/min. The mass spectrometer was operated at 70 eV in the electron impact mode. Phosphoric acid was quantified through comparison with an external standard curve. The standard curve was fit to the following linear regression model generated from a range of 0.045- to 0.81- μ g injections of phosphoric acid (Sigma-Aldrich):

$$y = 0.07313 + 0.0000017x$$

where x is the area under the peak, and y is the μ g of phosphoric acid injected ($r^2 = 0.98$).

A t -test was used to compare the means of phosphoric acid concentrations in worker fecal droplets and larval anal liquid.

Results and Discussion

Phosphoric acid was found in all samples. A typical chromatogram of larval anal liquid is shown in Fig. 1. Mass spectra of tris(trimethylsilyl) phosphate in larval anal liquid and the phosphoric acid standard are shown in Fig. 2. Phosphoric acid made up $2.13 \pm 1.08\%$ ($n = 11$) of the dry weight of worker fecal droplets and $2.09 \pm 0.72\%$ ($n = 5$) of larval anal liquid. There was no significant difference in phosphoric acid concentrations between worker fecal droplet and larval anal liquid ($t = -0.059$, $df = 14$, $P = 0.95$). The rate of phosphoric acid production in workers was 0.08 ± 0.024 μ g/ant/d.

Thus, phosphoric acid excretion by the red imported fire ants was shown, but excretion of other forms of phosphorus cannot be excluded, because phosphorus can be excreted in organic forms as well. Excretion of phosphate has been studied in other insects. For example, Kollien et al. (2001) studied the ionic composition of rectal contents of the reduviid bug, *Triatoma infestans*, and found that the concentration of phosphate increased within the first 1 or 2 d after feeding and then reached a level of ≈ 210 mM.

Phosphoric acid is commonly applied to promote plant growth (Hignett 1985). It is also a nutrient of

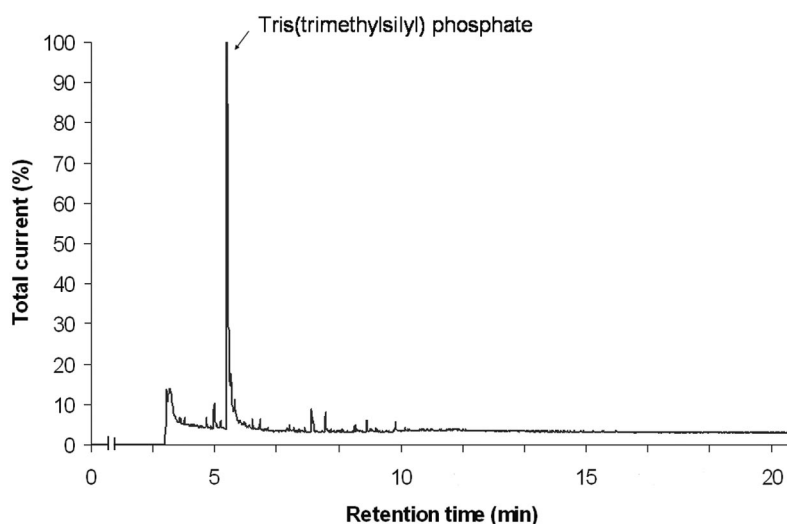


Fig. 1. Typical GC-MS total ion chromatograms of larval anal liquid.

yeast (King 1993). Phosphoric acid enrichment in mound soil may affect other biota in the ant nest by changing nutrient constituents. Additionally, phosphoric acid is an acidulant that can decrease the pH of mound material. Changing acidity can have a significant effect on behaviors of other chemical compounds, such as antimicrobial substances. The fact that ants produce antimicrobial compounds has been well documented, such as formic acid, D-3-hydroxydecanoic, indoleacetic, and phenylacetic acids (Hölldobler and Wilson 1990). Because the pH of the

environment determines the proportion of the dissociated and nondissociated forms of an organic acid near its pK_a , it has a significant effect on the antimicrobial activity of organic acids (Eklund 1983). Phosphoric acid may assist in retarding microbial growth in the mound of red imported fire ants by providing acidity. It may be important to the health of ant colonies by suppressing the growth of pathogenic microorganisms in the nest. However, it may cause difficulty in establishing populations of microbial control agents in ant colonies.

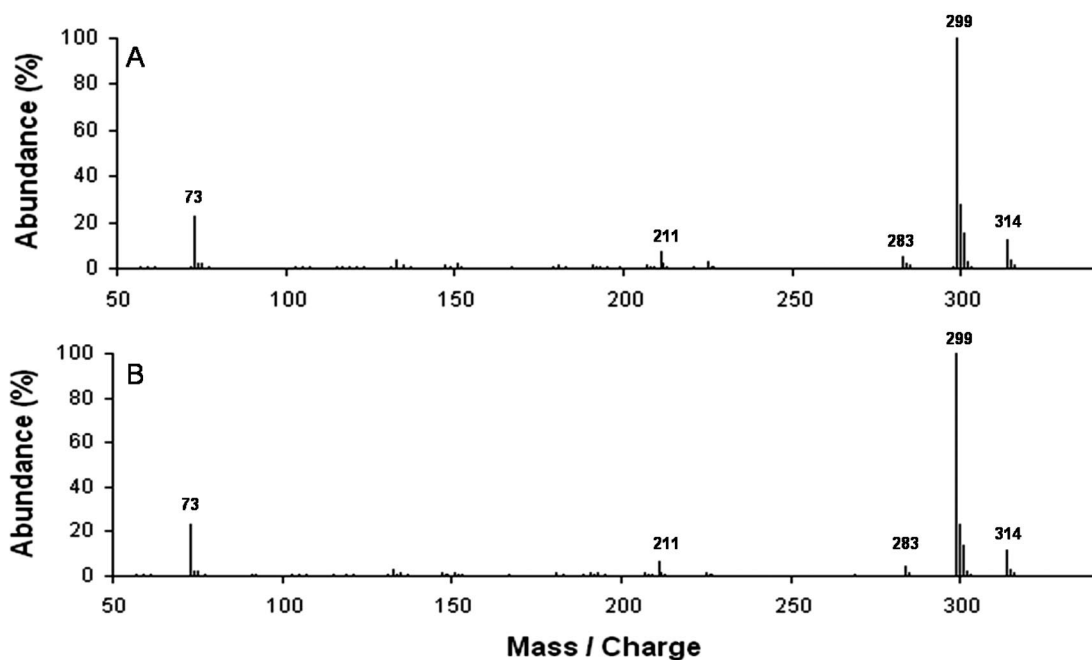


Fig. 2. Mass spectra of tris(trimethylsilyl) phosphate in larval anal liquid (A) and in phosphoric acid standard (B).

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